Cell Metabolism
Minireview
Focus on Brown Adipose Tissue

Distribution and Development of Brown Adipocytes in the Murine and Human Adipose Organ

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Murine white and brown adipocytes are found together in dissectible visceral and subcutaneous fat depots supplied by specific vessels and nerves, forming a multi-depot organ with plastic properties. Many of the anatomo-physiological features of murine fat depots apply to humans.

The recent demonstration of considerable amounts of metabolically active brown adipose tissue (BAT) in many adult humans has renewed the scientific interest in the subject worldwide, mainly because of its antiobesity effects, documented in murine models. Genetic ablation of BAT or of all β-adrenergic receptors, which are responsible for its activation, induces obesity in mice (Lowell et al., 1993; Bachman et al., 2002), whereas ectopic expression of UCP1—the protein-activating brown adipocyte thermogenesis uniquely found in BAT—in white adipose tissue (WAT) results in resistance to obesity (Kopecky et al., 1995). Accordingly, administration of drugs capable of increasing BAT activity curbs obesity and related disorders, such as type 2 diabetes, in animal models (Ghorbani and Himms-Hagen, 1997).

Here, the distribution of BAT is described in the framework of a concept based on rodent and human adipose tissues anatomy: the adipose organ (Cinti, 1999). The concept hinges also on plasticity, a physiological property that enables phenotype changes in the organ and offers new prospects for the treatment of obesity.

The Concept of Adipose Organ

Brown and white adipocytes have widely different morphologies, mainly in terms of the shape of the lipid droplets and of mitochondria. Lipids are organized as multiple, small droplets in brown adipocytes and as a single, large lipid droplet in white adipocytes (Figures 1A and 1B). Mitochondria are large and numerous and are endowed with laminar cristae in brown adipocytes (Figure 1C), whereas they are small and elongated, with randomly oriented cristae, in white adipocytes. These differences correspond to different functional roles—respectively, heat production with energy dissipation and energy storage and distribution. The function of brown adipocytes is critically related to UCP1, the mitochondrial protein uniquely expressed in this cell type and therefore considered as the molecular marker of BAT (Cannon and Nedergaard, 2004). Despite their different anatomy and functional roles, brown and white adipocytes are found together in dissectable structures, the fat depots (Figure 1D). In small rodents, a careful dissection discloses that subcutaneous and visceral depots have a distinct anatomy preserved at different ages and, in different genders, strains and experimental conditions. Each subcutaneous depot is surrounded by a connective capsule that provides a cleavage plane for dissection and is supplied by specific vessels and nerves, mainly noradrenergic.

The anterior subcutaneous depot is the most complex; its body has a pyramidal shape whose apex lies deep in the interscapular area. Symmetric lateral projections, mainly located under the scapulae and among the dorsal paravertebral skeletal muscles, extend to the cervical and axillary regions in close connection to large vessels at the base of the neck.

The posterior subcutaneous depot is found at the base of the hind legs. It is more compact and consists of a single band extending from the lumbar region (dorso-lumbar portion) to the inguino-crural (inguinal portion) and the pubic and gluteal regions (gluteal portion), merging with the contralateral depot at the level of the pubis. Subfascial depots are found in the limbs. In the hind legs, two main depots are located in the thigh and at the level of the popliteal fossa, respectively.

The visceral depots are found in the thoracic and abdominal cavities, where they are predominantly contained in areas delimited by serous membranes. The main thoracic (mediastinal) depot lies around the aorta and the proximal part of its thoracic branches. The abdominal depots are divided into retro- and intraperitoneal. The retroperitoneal depot par excellence has an elongated, conical shape; it extends longitudinally between the spine and the posterior abdominal wall in a paravertebral position and is separated from the perirenal depot by a peritoneal fold. Males also have epididymal depots. In females, perirenal, periovarian, parametrial, and perivesical fat are all found in the same depot (abdomino-pelvic depot).

The omental depot is small in rodents, but, as in humans, it is connected to the greater curvature of the stomach; the mesenteric depot is outlined by the two peritoneal leaflets holding the intestine against the posterior abdominal wall (see also the legend of Figure 1D).

The parenchyma of most subcutaneous and visceral depots in small rodents consists of both types of adipocytes organized into WAT and BAT, which are endowed with distinct vascular and nerve supplies. BAT is characterized by a denser network of capillaries and noradrenergic parenchymal fibers (Cinti, 1999).

Quantitative study of the composition of the parenchyma of the dissectible fat depots of adult Sv129 female mice (Murano et al., 2009) showed that the anterior subcutaneous depot is mainly composed of BAT (easily identified by its color in Figure 1D, animal kept at 28°C), whereas the posterior subcutaneous depot is predominantly made up of WAT, like the retroperitoneal and mesenteric depots. The mediastinal depot is mainly BAT, whereas the abdomino-pelvic depot is equally composed

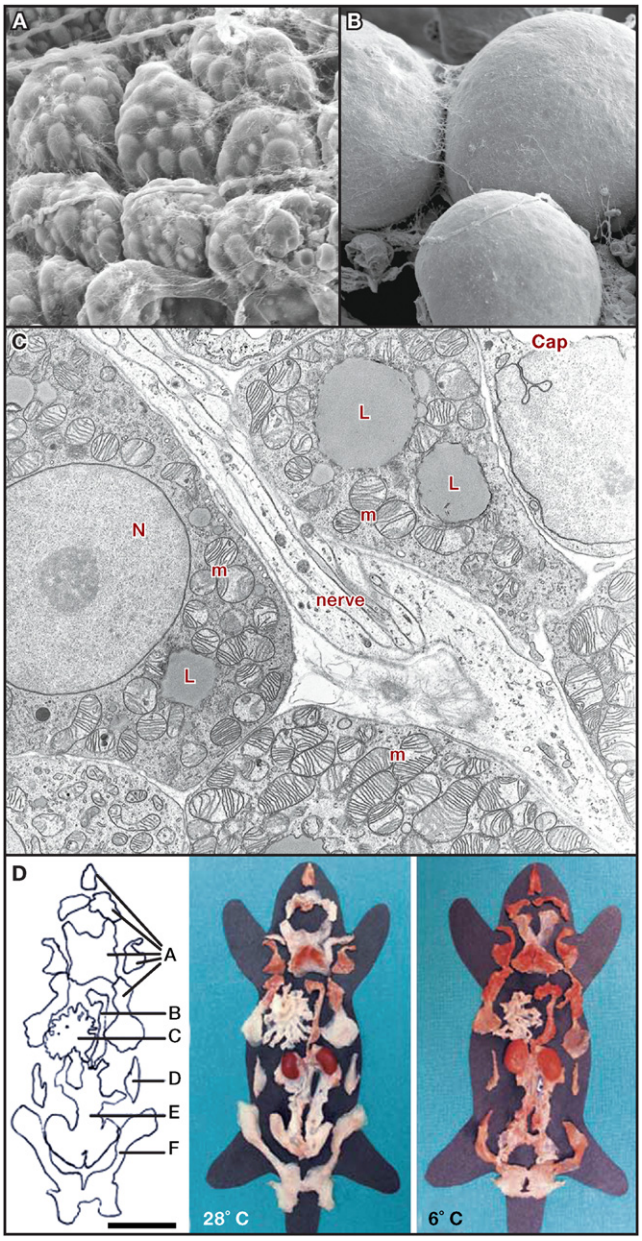

Figure 1. Brown Adipocyte Morphology in the Mouse Adipose Organ
(A and B) Scanning electron microscope image of brown (A) and white (B) adipocytes.
(C) Transmission electron microscopic appearance of BAT. Brown adipocytes with the cytoplasm filled with large mitochondria (some indicated as "m") and small lipid droplets (L). A parenchymal nerve can be seen among the adipocytes. N, nucleus; Cap, capillary.
(D) Gross anatomy of the adipose organ of adult female 129Sv mouse. (Middle) Mouse acclimatized to a warm temperature (28°C for 10 days). (Right) Mouse exposed to cold (6°C for 10 days). The different color of the organ is due to the increase in BAT and the decrease in WAT content. The organ is made up of two subcutaneous depots, anterior (deep cervical, superficial cervical, interscapular, subscapular, and axillo-thoracic; a) and posterior (dorso-lumbar, inguinal, and gluteal; f), and of several visceral depots: mediastinal (b), mesenteric (c), retroperitoneal (d), and abdomino-pelvic (perirenal, periovarian, parametrial, and perivesical; e).
Scale bars: (A) 15 μm, (B) 30 μm, (C) 2 μm, and (D) 1 cm.
(D) was reproduced with permission of New Century Health Publishers LLC, Coppell, TX, USA.

of WAT and BAT. Of note, in these mice, ~60% of the parenchyma of the whole adipose organ is made up of BAT at thermoneutrality. Our unpublished data suggest that the fat depots of B6 mice also have a mixed composition but with different WAT/BAT ratio.

In most adult mammals, WAT is prevalent in the adipose organ, but the WAT/BAT ratio varies with genetic background, sex, age, nutritional status, and environmental conditions (Figure 1D).

Such mixed composition of the fat depots, which contain the majority of the body fat (small amounts of white adipocytes are also found in organs such as bone marrow, skin, parotid glands, parathyroid glands, thymus, lymph nodes, and pancreas), prompts the question of why these two different cell types with different functional roles are found in the same organ.

Our data and findings by other groups support the concept that at least some adipocytes in each population can reversibly turn into one another. In basal conditions, the adipose organ uses the two tissues to meet the two physiological requirements of heat production (BAT) and energy storage (WAT). In the event of sustained heat requirement (i.e., chronic cold exposure) WAT can convert to BAT (Figures 2A and 2B), whereas, in case of chronic exposure to an "obesogenic environment," BAT is able to transform into WAT to store a greater amount of valuable energy molecules. Several data support the notion that this process occurs through direct transformation of adult cells, i.e., via physiological reversible transdifferentiation (Himms-Hagen et al., 2000; Granneman et al., 2005). The process involves not only genetic reprogramming of adult cells, but also tissue reorganization with changes in the density of capillaries and parenchymal nerve fibers (Murano et al., 2009).

However, it cannot be excluded that the brown adipocytes emerging in rodents in "classic" WAT depots in response to β-adrenergic receptor stimulation are the result of de novo differentiation of stem cells or committed brown preadipocytes, as also suggested by other researchers (reviewed in Gesta et al., 2007; Ravussin and Kozak, 2009). The fairly stable nature of white and brown preadipocyte cell lines in culture makes this hypothesis more likely than the transdifferentiation of WAT to BAT. However, the available data in in vivo models support the notion that certain preadipocytes found in WAT and mature white fat cells can be stimulated to undergo brown adipogenesis. Further studies are required to gain greater insights into the emergence of brown adipocytes in various fat depots.

Of note, white-to-brown-to-white transdifferentiation is not the sole example of the phenomenon in the adipose organ. Recent data from our laboratory highlighted the adipo-epithelial-adipo transformation of mammary gland adipocytes during pregnancy, lactation, and postlactation (Morroni et al., 2004; De Matteis et al., 2009).

Brown Adipose Tissue in Adult Humans

In humans, fat is also organized into subcutaneous and visceral compartments. The subcutaneous fat forms a continuous sheet around nearly all parts of the body. The mammary and gluteal regions are particularly fat rich in females. The human visceral depots are similar to those of the murine adipose organ, with the exception of epididymal fat, which men lack.

In adult humans, discrete areas containing metabolically active BAT have recently been identified by fluorodeoxyglucose

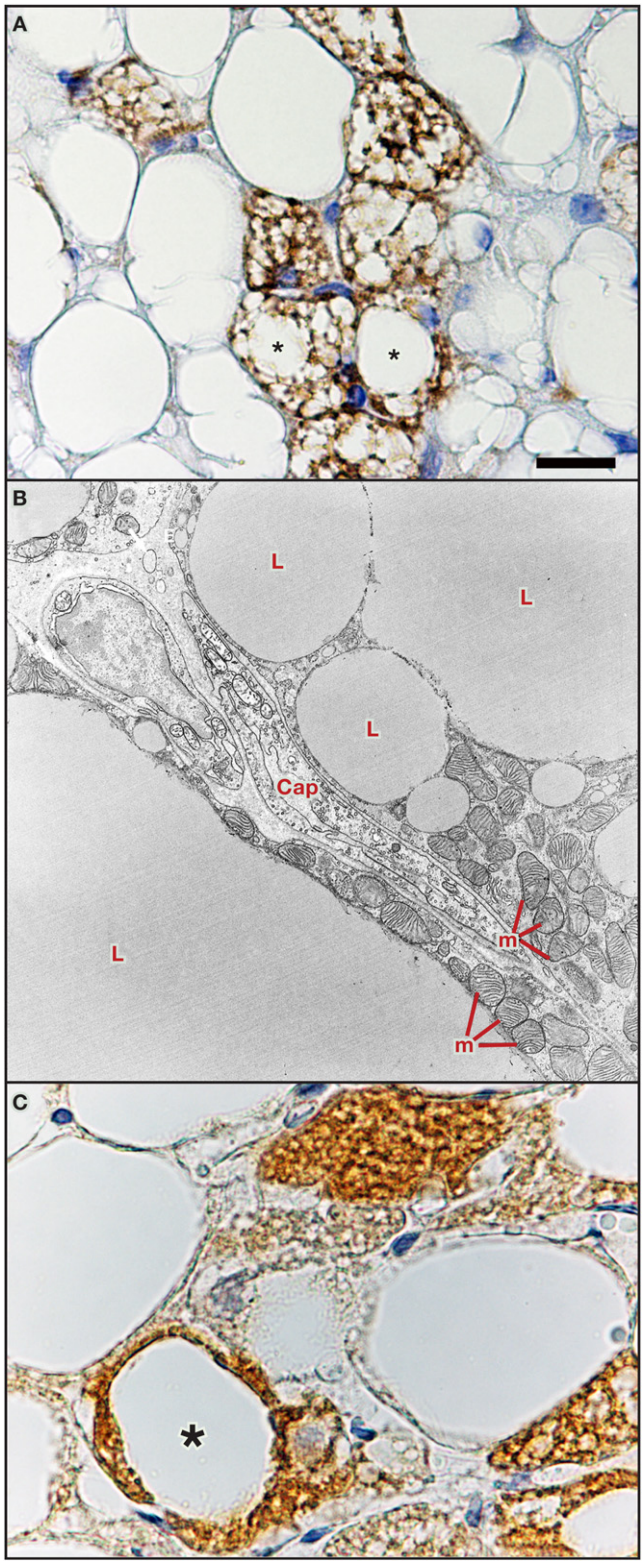

Figure 2. Paucilocular Adipocyte Morphology in White Fat Depots of Mice and Humans Subjected to Adrenergic Stimulation

UCP1-immunoreactive cells (brown color) with an intermediate morphology between white and brown adipocytes (*, paucilocular adipocytes) are found together with classic brown adipocytes (also brown) in WAT of mice and humans subjected to adrenergic stimulation.

(A and B) (A) Light microscopy and (B) transmission electron microscopy (EM) of inguinal WAT of cold-exposed mouse. Note the similar (BAT-like) EM features of mitochondria in the peripheral cytoplasmic rim of the paucilocular adipocyte shown in (B) (lower-left corner) to those of the classic mitochondria of the adjacent multilocular brown adipocyte (upper-right corner). L, lipid droplets; m, mitochondria; Cap, capillary.

(C) Light microscopic image of a UCP1-immunoreactive paucilocular adipocyte (*) in omental fat of a subject with pheochromocytoma. Adjacent UCP1-negative white adipocytes and classic multilocular UCP1-immunoreactive brown adipocytes.

Scale bars: (A) 30 μm, (B) 1.5 μm, and (C) 45 μm.

positron emission tomography (FDG PET) combined with computed tomography (CT). Several studies have documented that the metabolically active areas detected on PET-CT actually are UCP1-positive brown adipocytes (Virtanen et al., 2009; Cypess et al., 2009; van Marken Lichtenbelt et al., 2009) and have reached similar conclusions, i.e., that, irrespective of age and gender, metabolically active BAT is found in adult humans in the neck and upper-chest regions. Noradrenergic stimuli (i.e., cold exposure) can activate BAT and expand its PET-detectable signals in adult humans, ostensibly more often in women; the presence of BAT correlates inversely with body fat, especially in older subjects.

We found UCP1-immunoreactive brown adipocytes in perithyroid fat biopsies corresponding to the BAT area disclosed by PET-CT in about one-third of the adults who we examined. In these subjects, histological examination demonstrated mixed brown and white adipocytes and a vascular and nerve supply similar to that of the murine adipose organ. The areas predominantly containing brown adipocytes had a greater density of capillaries and noradrenergic fibers than those predominantly containing white adipocytes. The proportion of UCP1-positive adipocytes among all adipocytes in these individuals ranged from 3% to 31% (average 13.3%) (Zingaretti et al., 2009).

PET-CT scans of subjects with pheochromocytoma (Kuji et al., 2008) or with chronic exposure to cold (Saito et al., 2009), two conditions characterized by noradrenergic hyperstimulation, documented larger amounts of activated brown adipocytes. In either group of subjects, the anatomical distribution of BAT in the adipose organ was similar to the one described in small mammals, i.e., around the major vessels (the aorta and its main branches: carotids, subclavian, intercostal, and renal arteries), in relation to the need for distributing to the body the heat produced by BAT via the circulation. Of note, this anatomical distribution of BAT in adults is reminiscent of the human newborn pattern described many years ago (Merklin, 1974) and provides new relevance to several studies performed in the 1980s that described the detection of UCP1 mRNA and its translation product in different areas of the human adipose organ at all ages (Lean and James, 1986).

In brown fat islands examined by electron microscopy, we observed cells with discrete characteristics closely associated with capillaries. They were quite numerous (one in every 5–10 capillaries) and were characterized by a distinct basal membrane, glycogen particles, and numerous large mitochondria with several poorly differentiated cristae. Their similarity to the features described in rodent brown adipocyte precursors suggests that they are human brown adipocyte precursors (Zingaretti et al., 2009).

Benign neoplasms usually reproduce the features of the normal tissues from which they derive but with enhanced developmental characteristics. In a hibernoma (a benign BAT neoplasm) removed from the chest of a 17-year-old man, we recently observed cells whose electron microscopic features were identical to those described above for brown adipocyte precursors in normal BAT of adult humans. These cells also had a pericapillary location, but they were much more numerous (1 out of every 2 capillaries), in line with the enhanced developmental features of neoplasms (Manieri et al., 2009). Adult humans therefore have a reservoir of brown preadipocytes. Of note, many UCP1-immunoreactive adipocytes in normal BAT, in the hibernoma, and in fat from subjects with pheochromocytoma (Figure 2C) had intermediate features between white and brown adipocytes (paucilocular adipocytes), suggesting that the transdifferentiation phenomenon described in rodents could also take place in humans. This entails that a brown phenotype of the adipose organ may be inducible also in adult humans by promoting the proliferation and differentiation of brown fat cell precursors or by inducing white-to-brown fat transdifferentiation. Of interest, two master regulators of brown phenotype induction, PRDM16 and BMP7, have recently been identified (Seale et al., 2007; Seale et al., 2008; Tseng et al., 2008). With regard to this, we detected PRDM16-immunoreactive cells in supraclavicular BAT of adult humans (unpublished data).

In conclusion, several lines of evidence support the concept of a true adipose organ characterized by two parenchymal cell types organized into two tissues with different anatomy and functional roles, both involved in the use of highly energetic molecules and cooperating through their ability to transdifferentiate. These properties allow physiological reprogramming of the adipocyte genome, enabling differentiated cells to change phenotype and function and resulting in considerable organ plasticity. White-to-brown adipocyte transdifferentiation could offer new therapeutic prospects for obesity and related disorders, also in light of the demonstration of discrete amounts of metabolically active brown adipocytes in adult humans and similarities between the human adipose organ and that of small mammals. Therapeutic strategies to treat obesity might include pharmacologic interventions aimed at facilitating brown adipocyte maintenance, stimulation of the growth of pre-existing brown precursors, and induction of white-to-brown adipocyte transdifferentiation by acting on targets such as β-adrenergic or PPARγ receptors or on "new" molecular regulators such as PRDM16 or BMP7.

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